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Response of commercial and candidate lines of soybean (*Glycine max* L.) against root rot disease and its chemicals management

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ABSTRACT

Soybean (*Glycine max* L.) is imperative nitrogen fixing leguminous crop cultivate for foodstuff and nourishment. Root diseases caused by soil-borne pathogens are often main constrains in legume crop production among these pathogens *Macrophomina phaseolina* is major limiting factor. Numerous strategies have been suggested to manage this infection but not an iota of them has

been utterly effectual. So, present study was prformed to manage the disease by screening of diverse cultivars and different fungicides. Surveys were conducted in major soybean producing areas Chakwal, Rawalpindi, Jhang and Kasoor. Isolations were made from 3-5mm pieces of diseased plant parts of soybean and pathogen was identified as *Macrophomina phaseolina* on the basis of morphological characters. Branches of mature hyphae that assisted to identify isolated pathogen *M. phaseolina* arose at right and acute angle that is near to 45 degree to main hyphae. A septum in the branch near the point of origin was also observed. The colony color of *M. phaseolina* appeared as black and greyish Pathogenicity test for the *Macrophomina phaseolina* was done by usingcut stem inoculation technique. Deep and asymmetrical necrotic wounds were observed that were extended toward root surface and hypocotyls in soybean plants. Above-ground symptoms were observed to appear between 1 to 4 weeks before normal development till maturity. Lesions on the stem, roots, seeds and pods were caused by pathogen. Micro-sclerotia produced in the vascular bundles and in the tissues belongs to pith portion, giving a greyish black look to the sub-epidermal tissues of the diseased plant's stem. *M. phaseolina* was mass cultured on moistened rice to carry out screening experiment. Sick beds were prepared by applying mass cultured pathogen to the field. Twelve soybean cultivars were screened for their resistance against *Macrophomina phaseolina* by adopting mass culturing method used for sick bed preparation under field condition. Among twelve (12) soybean cultivars, two cultivars, Kharif-93 and Malakand-91 were shown highly resistant. One cultivar Williams-82 found as resistant against *Macrophomina phaseolina* disease. Three cultivars FS-95, FS-84 and Malakand-96 were reported moderately resistant. Two cultivar (FS-85 and NARC-2) and four cultivars (Swat-84, Fawal-1, Ajmeri-1 and FS-83) were found susceptible and highly susceptible disease reaction respectively. For *in vitro* evaluation of fungicides, Poisoned food technique was employed for the evaluation of fungicides in the laboratory. Four fungicides viz. Flumax, Hombre, Nonak and Maladydew were evaluated against *M. phaseolina* @ 50ppm, 100 ppm and 200 ppm of each treatment were used. Amongst the four incorporated fungicides the maximum percentage growth inhibition was observed in case of Flumax @ 200 ppm (T_4) (37.44%) followed by Nanok (29.668%) with 200 ppm (T_{13}) concentration. While minimum percent growth inhibition was observed in case of Hombre after control (T_1) (i.e., 0.00%). For field evaluation of fungicides the best evaluated fungicides (Flumax and Nonak) from *in-vitro* experiment were incorporated in the field experiment. The seeds were soaked in fungicide solution for twenty minutes then these seeds were dried for thirty minutes before sowing, while in control the seeds were soaked in distilled water. The treatments were applied on 2 moderately resistant varieties (FS-95 and Malakand-96). Treatment T1 (Flumax) gave best result as the maximum number of pods recorded (23 Pods for FS-95 and 22.4 Pods for Malakand-96).

1. INTRODUCTION

Soybeans are called "miracles golden bean" due to the high content of minerals (P, K, Ca and Zn) and proteins (42-45%) (Rahman *etal.,* 2011). In the US, soya is also used for bio-fuel production, because soya has a low nitrogen advantage, such as sunflower (Pimentel and Patz 2005; Benvindo Verde *et al*. 2018a & 2018b). Soybean is exposed to numerous diseases caused by bacteria, fungi, mycoplasma, nematodes and viruses (Sweets, 2008). *M. phaseolina* Goidanich is an imperative pathogen of soil-borne nature that is the reason of most important economic damage in soybean yield (El-Barougy *et al*., 2009 and Mengistu *et al*., 2007). The infection of charcoal rot, is supreme obvious for the duration of the reproductive stages of plant development, even though the fungi can also be isolated from infected roots of plants all over the developing period (Kendig *et al*., 2000). *Macrophomina phaseolina* (Tassi) Goidanich found over 500 plant species, including soybeans, corn and cotton (Wyllie, 1988). The charcoal root rot is one of the fourth most important disease of soybean along with cyst nematode, Phytophthora root and fungal rot disease (Doupnik, 1993). According to Sinclair and Backman (1989) in Soybean, the symptoms of unexpected rot appears in central measurements at high temperatures of (28-35 °C) and low humidity in the soil or environmentally damaging situations may give pressure to the plant. Even however primary contaminations occur in the developmental stage, they will continue to be dormant until the soya plants reach adulthood (Short *etal*., 1978). The most common diagnosis of rheumatic diseases is the intracellular tissues of the plants at the beginning of the dead stage, and the texture of the tissues is gray because of the production of micro-sclerotia in large quantity in vascular tissues. Incongruity soya sound control strategies include a reduction in the inoculation of pathogenic rotation pathogens to lessen plant populaces to stimulate quick and potent development of plants, planting and irrigation dates to alleviate stress (Todd, 1993). Researchers have investigated the effectiveness of soil fungal isolates which can be used in preventing or inhibiting pathogenic fungal spore growth and contamination, in addition to biological management methods, preventing host contamination or preventing the development of causing organisms (Siddiqui and Mahmood, 1993). If these strategies are not generally adequate control of this problem, the most effective toll against this disease is use of use of resistance/tolerant germplasm can be adopted (Bowen *et al*., 1989).

The identification of genetic resistance against *M. phaseolina* have not been achieved uptill now. However some available cultivars respond as tolerant against the infections by such pathogens. This indicates the existence of some resistance towards *M. phaseolina* (Pearson *et al*., 1984). The tolerant behavior of some cultivars with high yielding output and the lowest levels of root infections was observed by Short *et al*., in 1980. The current study will plan to observe the behavior of soybean cultures in a exceedingly infected field with *Macrophomina phaseolina* for the resistance and pathogen sensitivity. While information on the management of this pathogen is lacking on soy in the country, studies was planned with the following objectives.

- To assess the capability of pathogen to cause the disease in soybean
- To identify the soybean genotype with high root rot resistance
- To evaluate the best fungicide for the management of pathogen (*in-vitro* and *in-vivo*)

To achieve the above goals, the following line of work was done

- Disease samples were collected from different soybean cultivated fields.
- Cleaning of samples, Isolation, identification and multiplication of the M. phaseolina was done.
- Collection and sowing of soybeans germplasm, including hybrids and local varieties.
- Establishment of disease incidence.
- Evaluation of different fungicides for the management charcoal rots disease.

2. MATERIALS AND METHODS

2.1. Survey and collection of disease samples

During the cropping season of 2016-17 surveys were conducted in major soybean producing areas (Chakwal, Rawalpindi, Jhang and Kasoor) Soybean plant parts (lower stem, roots and soil) infected with micro-sclerotia of *Macrophomina phaseolina* were collected from infected farmers' fields and research areas of UAF and AARI. These samples were properly packed in paper bags and polyethylene bags, labeled and brought to Seed Health Testing Lab. The samples were stored at 4.0 ºC until processed for isolation and identification.

2.2. Isolation of *Macrophomina phaseolina*

Disease part of root were cut into small pieces of 5 to 7 mm. Sodium hypochlorite (NaOCl (0.1%)) was used for surface disinfection of these tiny pieces and then rinsed thrice in sterilized distilled water. The pieces were transferred to petri plates containing sterilized PDA media. These plates were incubated at 27±2°C for 3-5 days to obtain good fungal growth. For purification of fungal isolate a minor portion of the fastest growing colony of *Macrophomina phaseolina* was taken from the growing tip of fungal colony and spread on petri-dishes containing potato dextrose agar medium (Potato Starch 20g, Dextrose 20 g, agar 20 g and water 1000 ml) and incubated in dark at 25 ± 1ºC for 3-5 days.

2.3. Purification and identification of pathogen

A small portion of the colony containing sclerotia was placed in a drop of sterilized water on a slide and agitated with a sterilized needle to separate the sclerotia from the mycelia. Separated sclerotia were then placed on petri-dishes of 90 mm diameter containing PDA medium. Plates were incubated at 27±2°C for 3-5 days and identified on the basis of standard key (Barnett and Hunter, 1972).

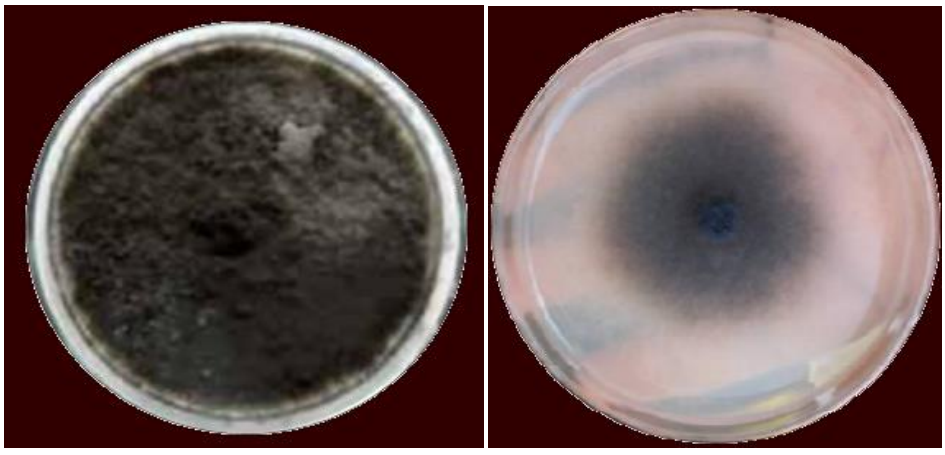

Figure 1 Young growing and mature culture of isolated pathogen

2.4. Preservation of pathogen

The purified fungal culture 5 mm disc from each isolate growing on PDA medium, using single sclerotia, was transferred to 10 ml culture tubes and incubated in dark at 25±1°C for 6 days, until the surface of PDA was covered with dense sclerotial layer of the fungal culture. The culture tubes were labeled and stored at 4 ºC.

2.5. Pathogenicity test

Pathogenicity of isolated fungus was tested using cut stem inoculation technique and in vivo sick pot method.

2.5.1. Cut stem inoculation technique

For the determination of the ability of the pathogen to cause the disease in the soybean crop pathogenicity test for the isolated pathogen *Macrophomina phaseolina* was carried out under lab condition. The plants were grown in the pots and inoculated at the age of four (04) weeks. Cut stem inoculation technique was adopted for the mentioned purpose (Twizeyimana *et al*., 2012). A cut of 2.5cm depth was given to four weeks old plants of soybean at the stem apex with the help of sharp razor blade. The open end of a 10- to 200-µl pipette tip (Fisher Scientific) was pushed into the margin of an actively growing *Macrophomina phaseolina* culture growing on potato dextrose agar, and a circular disc of fungal mycelium and agar was cut and removed. The pipette tip containing the agar disc with *Macrophomina phaseolina* mycelium was immediately placed over the cut stem and pushed down as far as possible in order to embed the stem into the medium and to secure the tip onto the stem. Three days after inoculation, the pipette tips were removed from each plant and discarded. Linear stem necrosis (in millimeters) and microsclerotia produced by *Macrophomina phaseolina* infection was observed.

Figure 2 (a & b) Soybean plants for pathogenicity test

2.6. Mass-culturing of *M. phaseolina*

Rice seeds were moistened and (1 ml water: 1 g rice seeds) and these were placed in conical flasks. Cotton plugs were used to close these flasks, aluminum foil was used to wrap and then was autoclaved at 121°C for 3 hours. After this cooling of flasks was done for 12 hours, and flasks were again autoclaved at 121°C for another 3 hours. After cooling, inoculation of seeds were done with a 5 mm diameter mycelial plug of fungus from a 7-day old culture of *M. phaseolina* and it was placed in incubator at 27±1°C for fifteen days.

The flasks were shaken at alternate days to obtain uniform colonization of the grains. These flasks containing inoculum were placed in refrigerator at 4°C until the use of inoculum in the field. The colonized seeds were plated on petri plates containing PDA media for confirmation of the fungus and were placed in an incubator for five days at 27±2°C.Mycelial growth was checked under stereoscope. The rice were air dried for 10 days at 24°C on a laboratory bench, and applied with seed at the rate of 1.5 g/m on planting.

2.7. Identifying soybean genotypes with high root rot resistance

A field experiment was conducted to assess the resistance levels in the promising varieties of soybean. The experiment was conducted under field condition in the plant pathology research area. During 2016- 2017, 12 varieties/lines were sown in research area of plant pathology UAF. Each cultivar/line was planted in a single row of 3 meter length, with plant to plant distance 30 cm. All the agronomic practices were followed except plant protection measures.

2.7.1. Sick Bed Preparation

Field plots were prepared in the research area of University of Agriculture Faisalabad. The soil was a Dexter fine-silt. Available soybean genotypes (FS-95, NARC-2, Kharif-93, Malakand-96, Malakand-91, Swat-84, FS-84, Williams-82, Fawal-1, Ajmeri-1, FS-85, FS-83) were checked for reaction to *Macrophomina phaseolina* infection. Sick bed was prepared in the research area of department of plant pathology. The mass cultured pathogen was applied to the field and seeds were sown.

Table 1 Disease rating scale

Disease Incidence	Quantitative Grade	Reaction
0 - < 5	Highly Resistant	HR
> 5 - < 10	Resistant	R
> 10 - <15	Moderately Resistant	MR
>15 - < 20	Susceptible	S
>20	Highly Susceptible	HS

2.8. *in-vitro* evaluation of Different Fungicides for their Effectiveness against *M. phaseolina*.

Different fungicides were tested for their efficiency to retard the fungal growth of *M. phaseolina in vitro* and through pot culture assay.

2.8.1. *In vitro* evaluation of fungicides

Poisoned food technique was employed for the evaluation of fungicides in the laboratory. Four fungicides viz. Flumax, Hombre, Nonak and Maladydew were evaluated against *M. phaseolina*. Three concentrations i.e., 50ppm, 100 ppm and 200 ppm of each treatment were used. The required quantity of fungicide was mixed with PDA at the time of pouring. Five replications were maintained for each fungicide for each of its concentration in CRD. The medium was shaken well so as to enhance proper mixing of the fungicides. To avoid bacterial contamination a little amount of streptomycin was added in each flask before plating; five mm disc was cut with the help of sterilized cork borer from seven days old culture of the test fungus and was placed in the center of the medium in the reversed position to maintain continuous contact of the pathogen with poisoned medium. PDA plates without fungicide served as control. The radial growth of the colony was measured when the growth in control plates reached the rim of the Petri plates. Percent growth inhibition under the influence of different fungicides was calculated on the basis of the control. Observation was recorded after control plate was completely covered with fungal growth. Percent inhibition of radial growth were calculated by the following formula.

$$\% \text{ Mycelial inhibition} = \frac{\text{Control (C) Area - Treated (T) Area}}{\text{Control (C) Area}} \times 100$$

Whereas

C = Diameter of fungus colony (mm) in control plate,

T = Diameter of fungus colony (mm) in treated plate.

Table 2 The doses of fungicides according to treatments

Treatment	Fungicide	Dose (ppm)
T1	Control	
T2	Flumax	50
T3	Flumax	100
T4	Flumax	200
T5	Hombre	50
T6	Hombre	100
T7	Hombre	200
T8	Maladydew	50
T9	Maladydew	100
T10	Maladydew	200
T11	Nanok	50
T12	Nanok	100
T13	Nanok	200

2.8.2. Field evaluation of fungicides

The best evaluated fungicides (Flumax and Nonak) from *in-vitro* experiment were incorporated in the field experiment. The seeds were soaked in fungicide solution for twenty minutes then these seeds were dried for thirty minutes before sowing, while in control the seeds were soaked in distilled water. Treated soybean seeds were sown in the field infested with root rot pathogen 5 g per meter of the row. All recommended agricultural practices were adopted. Root rot severity was recorded after 30 days of sowing. Plant growth parameters such as, plant height, number of pods and seed weight were recorded after harvest.

2.9. Statistical data analysis

Collected data were analyzed using Fischer's analysis of variance technique and the means were compared by least significance difference LSD test.

3. RESULTS AND DISCUSSION

3.1. Survey and sample collection

As a result of survey conducted to different localities, where soybean has been extensively cultivated, a large number of diseased samples were collected and plastic bags and brought to Seed Health Testing Lab., Department of Plant Pathology, University of Agriculture, Faisalabad. Samples in polythene bags were stored at 4 ºC, up till the seeds were incorporated in the following studies.

3.2. Isolation, identification and purification of fungi associated with diseased sample:

Isolation from diseased sample was done and different fungi associated with them were isolated and identified under microscope. Isolated fungi were *Macrophomina phaseolina*, *Rhizoctonia bataticola*, *Colletotrichum* spp. and *Alternaria* but most frequent fungus was *Macrophomina phaseolina*. So purification of *Macrophomina phaseolina* was done and preserved in test a tube at 4ºC. Identification was done on the basis of their morphological characters. The pathogen was identified as *Macrophomina phaseolina* on the basis of morphology; black color colony on nutrient medium, septation and branching pattern of fungal hyphae and sclerotia development.

3.2.1. Pathogenicity test performed through cut stem inoculation technique and in vivo sick pot method

Pathogenicity test was used to test the ability of pathogen to cause disease. To test the pathogenicity of *M. phaseolina*, cut stem inoculation technique and sick pot method used during study. Artificially inoculated soybean plant through cut stem inoculation technique showed linear necrosis. Seedling emerged from sick pot showed dark spots on the cotyledons (Fig. 3). The symptoms appeared on emerging hypocotyls of infected seedlings as circular to oblong, reddish-brown, lesions that may turn dark brown to black. The infected crop showed premature yellowing of leaves. Leaves of infected plants remain smaller than normal and subsequently turn yellow prior to wilting. The dead leaves of infected plant remain attached to the petiole for several days after death. Micro-sclerotia formed in the vascular tissues and in the pith, giving a greyish-black appearance to the sub epidermal tissues

of the stem. After the death of the plant, numerous, minute, pinhead-sized micro-sclerotia appeared, which could be seen when the epidermal tissue of the lower stems and roots peeled from the affected parts.

Figure 3 Microsclerotia of *M. phaseolina* under the epidermis

Deep and irregular necrotic lesions extended toward hypocotyls and root surfaces were observed in soybean (Ammon *et al.*, 1975). Bristow and Wyllie (1986) observed the aboveground symptoms to appear between 1 and 4 weeks before normal maturity. The pathogen causes lesions on the roots, stems, pods and seeds. From ground level upwards, superficial lesions, light brown to grey in colour, infrequently appear on the stem. A twin stem abnormality is usually observed in greenhouse infections. Foliar symptoms progress from top of the plant downwards. Leaves of infected plants remain smaller than normal and subsequently turn yellow prior to wilting (Gupta and Chauhan 2005).

Figure 4 Soybean plant showing necrotic symptoms

Figure 5 Necrotic symptoms observed on cotyledon

Figure 6 Healthy plants without showing necrosis

3.3. Identifying soybean genotypes with high root rot resistance

Twelve soybean cultivars were screened for their resistance against *Macrophomina phaseolina* by adopting mass culturing method used for sick bed preparation under field condition. Among twelve (12) soybean cultivars, two cultivars, Kharif-93 and Malakand-91 were shown highly resistant. One cultivar Williams-82 found as resistant against *Macrophomina phaseolina* disease. Three cultivars FS-95, FS-84 and Malakand-96 were reported moderately resistant. Two cultivar (FS-85 and NARC-2) and four cultivars (Swat-84, Fawal-1, Ajmeri-1 and FS-83) were found susceptible and highly susceptible disease reaction respectively.

Table 3 Per plant disease incidence (%) of each variety

Plant No.	FS-95	NARC-2	Kharif-93	Malakan-96	Malakand-91	Swat-84	FS-84	William-82	Fawal-1	Ajmeri-1	FS-85	FS-83
1	10.8	18.62	4.415	9.92	4.36	23.81	11.7	4.6	19.9425	30.76	22.89	21.39
2	8.86	18.82	3.865	10.64	4.23	22.22	12.05	3.5	19.94	25.25	22.54	32.15
3	9.97	17.48	4.005	10.64	4.25	20.64	12.5	3.78	23.05	30.75	22.09	20.59
4	10.69	18.725	3.44	9.185	4.17	20.63	11.33	2.65	23.185	25.25	12.15	32.87
5	10.69	17.49	4.915	10.25	4.37	23.74	11.86	5.6	20.0675	19.65	11.62	32.34
6	9.235	18.77	4.565	10.75	4.32	23.88	12.11	4.9	20.05	25.15	14.59	20.98
7	10.3	17.51	5.465	10.64	4.44	20.77	12.05	6.7	23.185	25.25	11.43	21.04
8	9.985	17.275	4.715	9.185	4.34	20.74	11.33	5.2	20.0675	30.75	23.26	32.87
9	11.33	18.81	4.18	10.25	4.27	25.39	11.86	4.9	20.0475	36.35	22.73	21.23
10	10.47	18.6	3.95	9.935	4.24	21.01	11.7	6.7	24.6975	25.25	11.89	21.39
11	9.285	18.72	4.445	10.42	4.32	20.88	11.94	5.2	20.3175	30.75	11.54	21.15
12	11.24	17.21	3.675	9.235	4.2	22.35	11.3	4.13	20.1825	25.25	12.18	32.9
13	10.69	18.63	3.395	11.195	4.17	22.36	12.33	3.67	21.5275	36.25	33.37	20.76

Table 4 Analysis of variance (ANOVA)

Source	DF	SS	MS	F	P
Block	12	96.5	8.042		
Varieties	11	10214.2	928.564	94.72	0.0000
Error	132	1294.0	9.803		
Total	155	11604.7			
Grand Mean = 14.898 CV = 21.02					

Table 5 LSD All-Pairwise Comparisons Test of DI (%) for Varieties

Varieties	Mean	Homogeneous Groups
Ajmeri-1	28.205	A
FS-83	25.512	B
Swat-84	22.186	C
Fawal-1	21.251	C
NARC-2	18.205	D
FS-85	17.868	D
FS-84	11.851	E
FS-95	10.273	E
Malaknd-96	10.173	E
Williams-8	4.733	F
Malaknd-91	4.283	F
Kharif-93	4.233	F

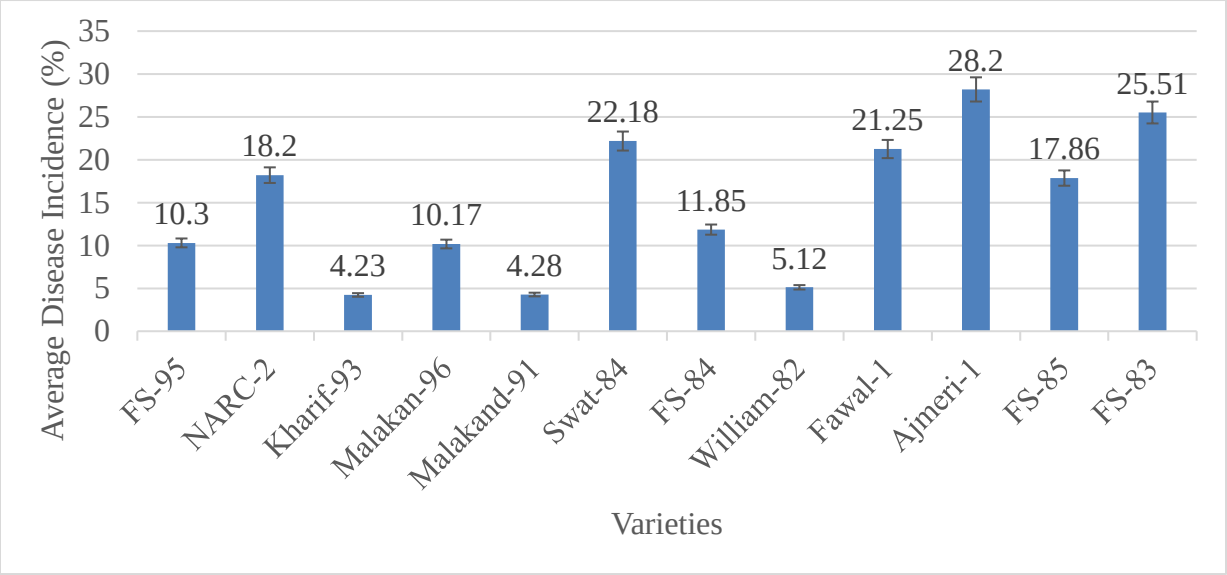

Figure 7 Graphical representation of variety-wise Disease incidence (%)

Table 6 Screening of soybean accessions to *Macrophomina phaseolina* infection

Variety		Disease score	Disease Incidence (%)	Diseases Reaction
FS-95	V1	3.9	10.35	Moderately Resistant
NARC-2	V2	6.4	18.2	Susceptible
Kharif-93	V3	0.4	4.23	Highly Resistant
Malakand-96	V4	3.5	10.17	Moderately Resistant
Malakand-91	V5	0.8	4.28	Highly Resistant
Swat-84	V6	8.1	22.18	Highly Susceptible

FS-84	V7	4.3	11.85	Moderately Resistant
Williams-82	V8	2.6	5.12	Resistant
Fawal-1	V9	7.6	21.25	Highly Susceptible
Ajmeri-1	V10	8.7	28.205	Highly Susceptible
FS-85	V11	5.9	17.86	Susceptible
FS-83	V12	8.4	25.51	Highly Susceptible

3.4. *In vitro* evaluation of fungicides against *Macrophomina phaseolina*

3.4.1. Poisoned Food Technique

Four fungicides were tested against *Macrophomina phaseolina* and the inhibition percentage was recorded as follows:

$$\% \text{ Mycelial inhibition} = \frac{\text{Control (C) Area - Treated (T) Area}}{\text{Control (C) Area}} \text{ X } 100$$

Table 7 CRD ANOVA for fungal inhibition (%)

Source	DF	SS	MS	F	P
Treatment	12	10715.06	892.965	470	0.0000
Error	52	98.7	1.899		
Total	64	10814.3			
Grand Mean = 14.696 CV = 09.38					

The *p-value* showed that the results were highly significant and this indicated that at least one of the applied treatment has no same effect. So to investigate which treatment performed better the LSD pairwise comparison test was performed. The results were interpreted on the basis of statistical analysis.

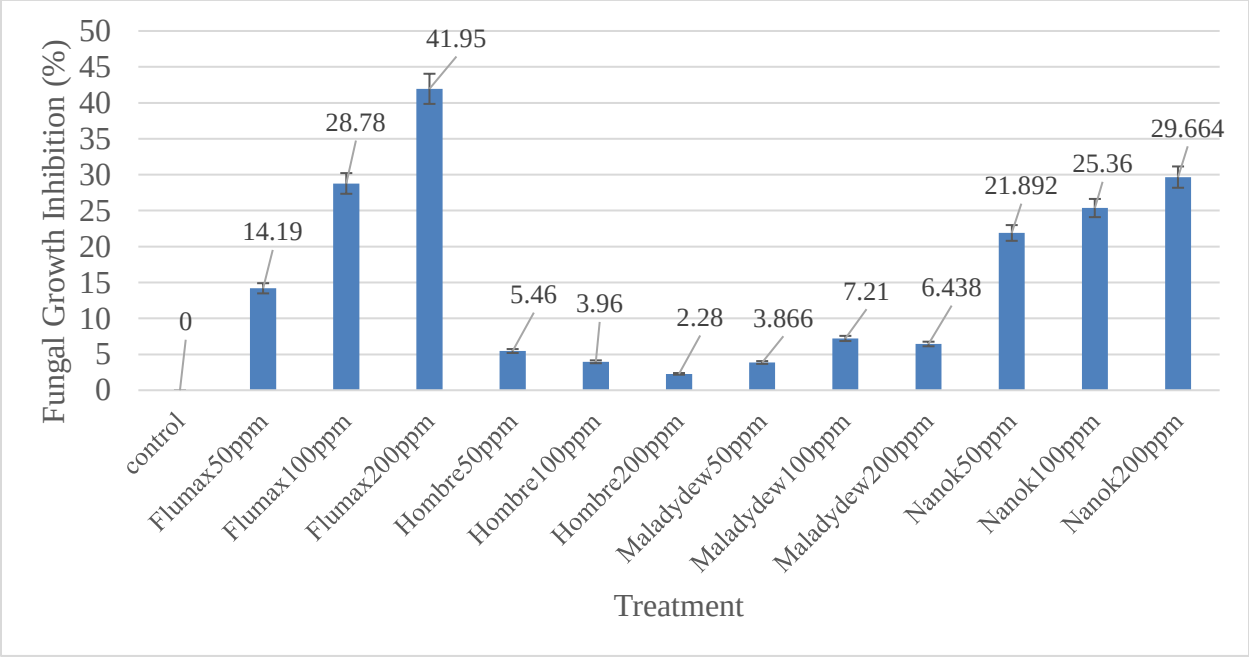

Figure 8 Graphical display of comparison between treatments' means

Table 8 LSD All-pairwise comparisons test of inhibition (%) by treatments

TREATMENT	Mean	Homogeneous Groups
T4	41.950	A
T13	29.664	B
T3	28.780	B

T12	25.358	C
T11	21.892	D
T2	14.190	E
T9	7.2100	F
T10	6.4380	FG
T5	5.4600	GH
T6	3.9600	HI
T8	3.8660	HI
T7	2.2800	I
T1	0.0000	J
Critical Value for Comparison = 1.7487		

Amongst the four incorporated finguicides the maximum percentage growth inhibition was observed in case of Flumax @ 200 ppm (T_4) (37.44%) followed by Nanok (29.668%) with 200 ppm (T_{13}) concentration. While minimum percent growth inhibition was observed in case of Hombre after control (T_1) (i.e.,0.00%).

3.5. Field Evaluation of fungicides

3.5.1 Seed treatment

Two fungicide (Flumax and Nanok) that showed best results in lab experiment were incorporated in the further studies. Seeds were treated with fungicides and sown under field condition during growing season. Data was recorded and evaluated on the basis of number of pods per plant. The treatments were applied on 2 moderately resistant varieties (FS-95 and Malakand-96) that were screened out in the screening experiment. The seed was dipped in fungicide solution for 2-3 hours before sowing then air dried under shade. Treatment T1 (Flumax) gave best result as the maximum number of pods recorded (23 Pods for FS-95 and 22.4 Pods for Malakand-96).

Table 9 RCBD ANOVA for effectiveness of treatments under field condition

Source	DF	SS	MS	F	P
BLOCK	4	20.33	5.0833		
TREATMENT	5	323.467	64.6933	23.58	0.0000
ERROR	20	54.867	2.7433		
Total	29	398.667			
Grand Mean = 18.667 CV = 8.87					

Table 10 LSD All-pairwise comparisons test of no. of pods by treatments

TREATMENT	MEAN	HOMOGENEOUS GROUPS
V1T1	23.000	A
V2T1	22.400	A
V1T2	19.000	B
V2T2	18.200	B
V1T3	15.000	C
V2T3	14.400	C
V1 = FS-95, V2 = Malakand-96, T1 = Flumax, T2 = Nanok, T3 = Control		
Alpha 0.05 Standard Error for Comparison 1.0475		
Critical T Value 2.086 Critical Value for Comparison 2.1851		

4. CONCLUSION

Among twelve (12) soybean cultivars, two cultivars, Kharif-93 and Malakand-91 were highly resistant. One cultivar Williams-82 found as resistant against *Macrophomina phaseolina* disease. Three cultivars FS-95, FS-84 and Malakand-96 were reported moderately

resistant. Two cultivar (FS-85 and NARC-2) and four cultivars (Swat-84, Fawal-1, Ajmeri-1 and FS-83) were found susceptible and highly susceptible disease reaction respectively.

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Conflict of Interest:
The authors declare that there are no conflicts of interests.

Peer-review:
External peer-review was done through double-blind method.

Data and materials availability:
All data associated with this study are present in the paper.

REFERENCE

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